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IgG4 anti-neurofascin155 antibodies in chronic inflammatory demyelinating polyradiculoneuropathy: Clinical significance and diagnostic utility of a conventional assay

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ABSTRACT

We aimed to validate the diagnostic utility of enzyme-linked immunosorbent assay (ELISA) for the detection of anti-neurofascin (NF) 155 antibody in 191 patients with chronic inflammatory demyelinating polyneuropathy (CIDP). Human NF155-based ELISA clearly distinguished between anti-NF155 antibody-positive and -negative sera. Fifteen CIDP patients (8%) were IgG4 anti-human NF155 antibody-positive, which were confirmed by western blot, cell-based assay and immunohistochemical study. None of disease controls or healthy subjects had positive results. Clinical presentation of IgG4 anti-NF155 antibody-positive patients was consistent with those in previous reports. This ELISA combined with determination of the IgG4 subclass is useful in screening for anti-NF155 antibodies.

1. Introduction

Neurofascin (NF) 155 is a glial protein that is one of the complex elements of septate-like junctions that anchors myelin loops to axons at paranodes (Stathopoulos et al., 2015). Autoantibodies against NF155 (anti-NF155 antibodies) have recently been identified in patients with combined central and peripheral demyelination (Kawamura et al., 2013; Ogata et al., 2016), and in a subset of patients with chronic inflammatory demyelinating polyneuropathy (CIDP), in whom IgG4 antibodies are predominant (Querol et al., 2014). IgG4 anti-NF155 antibody-positive CIDP patients have several characteristic clinical features, including younger onset, disabling tremor, sensory ataxia, higher frequency of distal dominant phenotype, higher protein levels in cerebrospinal fluid (CSF), and demyelination of central nervous system (Querol et al., 2014; Ogata et al., 2015; Devaux et al., 2016). Intravenous immunoglobulin therapy (IVIG) has little efficacy, but oral prednisolone, immunosuppressants, and rituximab are effective in patients with anti-NF155 antibody-positive CIDP (Ogata et al., 2015; Querol et al., 2015). These observations suggest that this condition is clinically homogeneous. However, the optimal treatment remains unclear, mainly due to the low frequency of cases with anti-NF155 antibodies. To establish this treatment, more information on anti-NF155 antibody-positive CIDP is needed, and this requires a convenient and cost-effective assay for initial screening for disease-related anti-NF155 antibodies.

In this study, we identified anti-NF155 antibodies with an enzyme-linked immunosorbent assay (ELISA) using human recombinant NF155 and confirmed the findings with western blotting (WB), cell-based assay (CBA) and immunohistochemistry (IHC) using rat sciatic nerves. Clinical features and treatment of anti-NF155 antibody-positive

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patients with CIDP were also analyzed and were compared with data from other laboratories, in order to examine whether ELISA is useful in initial screening for anti-NF155 antibodies.

2. Materials and methods

2.1. Patients and controls

Sera from 191 patients with CIDP were collected from teaching hospitals in Japan. These 191 Japanese patients were not included in previous two studies (Ogata et al., 2015; Devaux et al., 2016). The diagnosis of CIDP was made by attendant physicians according to European Federation of Neurological Societies/Peripheral Nerve Society (EFNS/PNS) guidelines (Van den Bergh et al., 2010). Sera from 16 patients with multiple sclerosis (MS) according to revised McDonald criteria (Polman et al., 2011), 57 patients with Guillain-Barré syndrome (GBS) (Asbury and Cornblath, 1990), 26 patients with Fisher syndrome (FS) characterized by ophthalmoplegia, ataxia, areflexia and little weakness (Fisher, 1956), and 20 healthy controls (HC) were also evaluated. Clinical information for CIDP patients was collected from clinical records and questionnaires sent to attendant physicians, and analyzed retrospectively. The diagnosis of distal acquired demyelinating symmetric (DADS) neuropathy was defined by fully or almost fully-preserved muscle strength of proximal muscles and distal dominant symmetric muscle weakness of extremities (differences in muscle strength by one or more Medical Research Council (MRC) scales compared with proximal muscles). Patients were considered responsive to the treatment if improvements in muscle weakness (one or more MRC scales) were observed within one month after administration. Treatment outcome was defined as favorable in patients whose Modified Rankin scale (mRS) scores at the last visit were decreased by one or more grade compared with those at diagnosis. Disease duration was defined as periods between the time of onset of symptoms and the time of last visit. Written informed consent was obtained from each subject. The study was approved by the Ethics Committee of the National Defense Medical College.

2.2. Detection of anti-NF 155 antibodies

2.2.1. Enzyme-linked immunosorbent assay

Recombinant human NF protein which corresponds to isoform NF155 (R&D Systems, Minneapolis, MN, USA) and recombinant rat NF protein (R&D Systems) were used as antigens. Polystyrene ELISA plates (96-well, Corning Life Sciences, Lowell, MA, USA) were coated with recombinant NF protein or phosphate buffered saline (PBS as control) overnight. After blocking, patient serum diluted 1:200 with 1% bovine serum albumin (BSA) in PBS was added as primary antibody and left to stand for 120 min. After washing three times, horseradish peroxidase-conjugated anti-human IgG-Fc antibody (MP Biomedicals, Solon, OH, USA) diluted to 1:500 with 1% BSA in PBS was added as secondary antibody and left to stand for 90 min. The wells were washed again and the chromogenic reaction was developed with *O*-phenylenediamine in phosphate-citrate buffer. Optical density (OD) was measured at 490 nm (Bio-Rad Laboratories Inc., Hercules, CA, USA). The corrected OD was calculated in duplicate by subtracting the OD values of PBS as the control.

For sera found to be positive, the subclass of IgG was examined using horseradish peroxidase-conjugated anti-human IgG1, IgG2, IgG3, IgG4 antibodies (IgG1 and IgG4: Life Technologies, Eugene, OR, USA; IgG2 and IgG3: Invitrogen, Carlsbad, CA, USA) as secondary antibodies. In addition, for determination of anti-NF155 antibody titers, ELISA was performed with multiplicative serum concentrations.

2.2.2. Western blot analysis

Recombinant human NF protein was mixed with sample buffer and boiled for 5 min. Antigen solution was loaded (250 ng/well) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. After SDS-PAGE, proteins on the gel were electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane (Merck Millipore, Darmstadt, Germany). After blocking, the PVDF membrane was incubated in patient serum diluted to 1:200 or monoclonal mouse anti-NF155 antibody (R&D Systems) diluted to 1:100,000 with blocking buffer overnight at 4 °C. The membrane was washed three times and incubated with horseradish peroxidase-conjugated anti-human IgG-Fc antibody (MP Biomedicals) diluted 1:5000 or anti-mouse IgG/IgA/IgM antibody (KPL, Gaithersburg, MD, USA) diluted 1:10,000 with blocking buffer for 1 h. The membrane was washed again and a band (around 120–140 kDa in reducing conditions) was revealed with LAS3000 (Fujifilm, Tokyo, Japan) through an enhanced chemiluminescence reaction.

2.2.3. Cell-based assay

Flow cytometric assay was performed as reported elsewhere Kawamura et al., 2013. In brief, NF155-turbo GFP-transfected and naive HEK293 cells were evenly mixed. Serum samples (2.5 μl) were added to 47.5 μl of cell-containing solution (1:20 dilution). After incubation at 4 °C for 60 min, cells were washed and bound IgG was detected with Alexa 647-labeled anti-human IgG antibodies (Life Technologies, Carlsbad, CA), diluted 1:500. After incubation at 4 °C for 60 min, cells were washed and analyzed by MACSQuant Analyzer (Miltenyi Biotec, Bergisch Gladbach, Germany). The mean fluorescence intensity (MFI) of cell-associated turbo GFP and Alexa 647 was measured for each sample. The MFI ratio was calculated by dividing Alexa 647 MFI of NF155-transfected cells by Alexa 647 MFI of NF155-untransfected cells, and delta MFI was calculated by subtracting Alexa-647 MFI of NF155-untransfected cells from Alexa 647 MFI of NF155-transfected cells.

2.2.4. Immunohistochemical study

Sciatic nerves were dissected from adult Sprague–Dawley rats and frozen immediately after dissection. Longitudinal cryostat sections (8 μm) were fixed for 5 min in acetone just before immunohistochemical staining. After blocking with 10% normal goat serum (NGS) in PBS for 30 min, double immunostaining was performed using rabbit polyclonal anti-contactin associated protein 1 (Caspr) antibody (Abcam, Cambridge, U.K.) and patient serum (diluted 1:100 and 1:160 with 10% NGS in PBS, respectively) as primary antibody. After 12 h, sections were washed three times with PBS for 30 min and incubated for 90 min with fluorescein isothiocyanate (FITC)-labeled anti-rabbit IgG antibody and rhodamine-labeled anti-human IgG antibody (Merck Millipore, Darmstadt, Germany) (both diluted 1:100 with 10% NGS in PBS) as secondary antibody. After washing again, sections were mounted with Fluoromount-G (Southern Biotech, Birmingham, AL, USA). Images were captured using a fluorescence microscope (Nikon, Tokyo, Japan).

2.3. Statistical analysis

A two-tailed Mann-Whitney *U* test was performed for continuous data for clinical features and a Fisher exact test was used to compare categorical data. $p < 0.05$ was considered to indicate significance. All analyses were performed using JMP Pro 11 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Detection of anti-NF155 antibodies

ELISA using human NF155 showed that, sera from 15 of 191 patients (8%) with CIDP had distinct reactivity (corrected OD from 0.331 to 1.358) that was clearly separable from those of the other 176 patients (Fig.1A). The IgG subclass of anti-human NF155 antibodies in these 15 patients was IgG4-predominant. None of the 99 disease controls and